Extracellular proteases of *Botrytis cinerea* and peculiarities of their secretion

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Numerous extracellular enzymes and metabolites of ubiquitous necrotrophic plant pathogen *B. cinerea* are shown to be true pathogenicity factors. However, some secreted enzymes of this fungus, such as proteases, are only potentially involved in infection process. In spite of extensive molecular-biological studies on extracellular proteases of *B. cinerea*, there is a clear evidence of limited information on their metabolic significance and even less is known about the regulation of their secretion, which could facilitate the selection of rational biocontrol methods against causal agent of grey mould disease. Our study has focused on complex biochemical and functional analysis of proteolytic activity of *B. cinerea* and thus promotes the evaluation of potential roles of its exoproteases.

Results from analysis of substrate specificity and inhibitor assay have revealed the prevalence of serine proteases in culture liquid of *B. cinerea*. The ability of *B. cinerea* to secrete at first (on 4^{th} dpi) aminopeptidases and subtilisin-like and later (on 6^{th} dpi) trypsin-like proteases at ambient pH 6,5 and 5,0, respectively, has also been demonstrated. Given that the highest aminopeptidase and subtilisin-like activities were coincided with the maximum of the total proteolytic activity at near-neutral pH values of the medium, it is possible to suggest the pH-dependent inactivation of early secreted proteases during the following cultivation accompanied by rather strong acidification. Conversely, the secretion of trypsin-like proteases, exhibiting activity under acidic conditions (pH 4,3 – 5,0), was sustained on significant level up to the end of cultivation. Extracellular subtilisin- and trypsin-like activities of *B. cinerea* are not considered to be exogenously regulated by its own inhibitors since no secreted trypsin or subtilisin inhibitory activities were observed.

The present work provides evidence that pH-, time course and biomass accumulation dependent regulatory mechanisms are essential for protease activity of *B. cinerea*. The data also demonstrated that aminopeptidases and subtilisin-like proteases are presumably involved in initial steps of plant cell colonization, whereas trypsin-like proteases may represent more specific enzymes acting during the later stages of infection process at acidic pH values, which are known to be often encountered in host tissues.